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CO-ADAPTATION MECHINISMS IN PLANT-NEMATODE SYSTEMS

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The review is aimed to analyze the biochemical and immune-breaking adaptive mechanisms established in evolution of plant parasitic nematodes. Plant parasitic nematodes are obligate, biotrophic pathogens of numerous plant species. These organisms cause dramatic changes in the morphology and physiology of their hosts. The group of sedentary nematodes which are among the most damaging plant-parasitic nematodes cause the formation of special organs called nematode feeding sites in the root tissue called syncytium (cyst nematodes, CN; *Heterodera* and *Globodera* spp.) or giant cells (root-knot nematodes, RKN; *Meloidogyne* spp.). The most pronounced morphological adaptations of nematodes for plant parasitism include a hollow, protrusible stylet (feeding spear) connected to three esophageal gland cells that express products secreted into plant tissues through the stylet.

Several gene products secreted by the nematode during parasitism have been identified. The current battery of candidate parasitism proteins secreted by nematodes to modify plant tissues for parasitism includes cell-wall-modifying enzymes, multiple regulators of host cell cycle and metabolism, proteins that can localize near the plant cell nucleus, potential suppressors of host defense, and mimics of plant molecules. Plants are usually able to recognize and react to parasites by activating various defense responses. When the response of the plant is too weak or too late, a successful infection (compatible interaction) will result. A rapid and strong defense response (e. g. due to the presence of a resistance gene) will result in the resistant (incompatible) reaction. Defense responses include the production of toxic oxygen radicals and systemic signaling compounds as well as the activation of defense genes that lead to the production of structural barriers or other toxins.

Key words: plant-parasitic nematodes, sedentary parasites, nematode secretions, co-adaptation mechanisms, resistance.

МЕХАНИЗМЫ КОАДАПТАЦИИ В СИСТЕМЕ РАСТЕНИЯ-НЕМАТОДЫ

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Фитопаразитические нематоды — облигатные биоторофные патогены, поражающие многие виды растений. Эти организмы вызывают резкие изменения в морфологии и физиологии своих хозяев. Седентарные нематоды, которые относятся к числу самых вредоносных видов паразитических нематод, формируют в тканях корня специальные структуры, названные питающими сайтами, — цистообразующие нематоды формируют синцитий (*Heterodera* и *Globodera* spp.), а галловые (*Meloidogyne* spp.) — гигантские клетки. Основными морфологическими адаптациями к паразитизму у фитонематод является стилет, приспособленный для проникновения через клеточную стенку растения, а также железы пищевода (одна дорсальная — ДЖ и две субвентральные — СВЖ), секреты которых выделяются в ткани растений через стилет. Охарактеризовано большое количество генов, которые кодируют белки, выделяемые нематодами в ткани растений, и установлена их функция. Секреторные выделения включают в себя ферменты, модифицирующие клетки хозяина, регуляторы клеточного цикла и метаболизма, ферменты, мимикрирующие белки растений, а также белки ядерной локализации. Растения способны распознавать и реагировать на паразитов, активируя различные реакции защиты. Незначительная или поздняя реакция растений на нематод приводит к поражению растений (совместимое взаимодействие). Быстрый и сильный защитный ответ, который может быть связан с присутствием у растений определенных генов, приводит к устойчивости (несовместимое взаимодействие). Защитный ответ связан с образованием токсических кислородных радикалов и системных сигнальных соединений, а также с активацией защитных генов, продукты которых приводят созданию структурных барьеров или токсических соединений.

Ключевые слова: фитопаразитические нематоды, седентарные паразиты, секреция нематод, механизмы коадаптации, устойчивость, гены устойчивости.

INTRODUCTION

Nematodes are one of the most successful groups of animals; they are diverse and abundant in almost all the soil, freshwater, and marine habitats. These small vermiform organisms occupy several trophic levels and play an important role in soil ecosystems. Among 20 000 known nematode species, approximately 20 % (4000 species) are associated with plants. Any studied plant species, either cultivated or wild, is a host for plant parasitic nematodes (PPNs).

The effects of a parasite on its host include mechanical damage, chemical influence, and the use of host nutrient resources. Parasitism is a form of antagonistic symbiosis of two heteronymic organisms forming a host- parasite system (HPS) in which one actor (parasite) puts on its host the regulation of mutual relations with the environment; the parasite feeds at its host's expense and frequently uses the latter as a shelter; the host does not benefit from the association and is often harmed by it causing host defense responses (Lebedev, 1995; Combes, 2005). Damage caused by a parasite is usually in the focus of attention in host- parasite relationships (HPRs). One of the main trends in the evolution of parasitism is to diminish the HPRs' antagonism. It is very important for the parasite not only to survive but also be able to complete its life cycle without severe damage to the host whose death leads to a destruction of the parasite itself.

The host may either prevent the parasite entry or suppress the development of the penetrated parasite killing it by immune responses. The aim of our review is to analyze modern data on adaptive mechanisms established in the evolution of PPNs that used different organs and tissues of plant hosts.

All PPNs are obligate parasites feeding exclusively on the contents of living plant cells. They are among the most damaging and uncontrollable pests of cultivated crops causing more than $US 100—125 billion economic losses in world agriculture (Bird, Kaloshian, 2003; Chitwood, 2003).

Many of them are polyphagous, non-specific to the particular plant host species range parasites; the oligophagous and monophagous plant parasites occur as well. Depending on plant organs they prefer to use for feeding, they may be classified into the root, stem and leaf phytonematodes. Less frequently, nematodes occur in flowers and fruits. Majority of PPNs attack roots, and, less often, other organs and tissues. Some PPNs are ectoparasites, living outside their hosts. These species cause severe root damage, some of them are important vectors of nepo- and tobraviruses (Brown et al., 1995). Other species feed inside roots as migratory or sedentary endoparasites.

The size of PPNs varies from 0.3 to 8 mm, mostly not exceeding 2 mm. Their bodies are always round in cross-section, non-segmented, threadlike or spindle-shaped; less often, in sedentary or slow-moving species of nematodes the body is pear-shaped or spherical (root-knot nematodes of the family Meloidogynidae and cyst nematodes of the family Heteroderidae). In both root-knot and cyst nematodes adult males are vermiform.

PPNs exhibit a high rate of motility and development of the trophic, reproductive, excretory, and nervous systems. Nematodes show the complex behavior during infection and fine host perception. As is typical of the phylum Nematoda, they possess a central nervous system and anterior chemosensory organs called amphids. Chemosensory signals are important for nematode search of host roots and also for a choice of appropriate penetration site on the host root or stem, and initiation of specialized feeding site by the infective juvenile (Perry, Moens, 2011).

The presence of special structures (the stylet and pharyngeal secretory glands used in parasitic feeding) is a characteristic feature distinguishing PPNs from free-living soil inhabiting nematodes. The stylet is a 12—28 μm hollow and very fine structure. Its inner capillary is narrow; only liquid nutrients may pass through it into the nematode pharyngeal duct. The capillary lumen of the stylet ensures a sufficient suction force of the organ which works like a syringe. The key organs used by the nematode for feeding on the cell contents of plant tissues are three secretory glands of the pharynx: the dorsal gland (DG) located in the spinal part of the pharynx, and two subventral glands (SVG) at its sides. Each secretory gland is a single cell containing the large nucleus, the Golgi system, the endoplasmic reticulum, and secretory granules. The structure and functions of secretory glands have been analyzed in detail (Anderson, Byer, 1975; Endo, 1984; Hussey, 1989; Hussey, Mims, 1990; Hussey et al., 2002).

Plant parasitic nematodes are migratory ectoparasites or endoparasites. Migratory parasites move through the root intracellulary, causing massive cellular necrosis. The most economically important groups of nematodes are sedentary endoparasites which include the genera *Heterodera* and *Globodera*, and the root-knot nematodes of the genus *Meloidogyne* (Williams, Hussey, 1996).

STRUCTURAL FEATURES OF THE PLANT CELL RESPONSE TO SEDENTARY NEMATODES

Sedentary nematodes cause characteristic formations on roots. Root-knot nematodes (RKNs, *Meloidogyne* spp.) induce galls or root-knots. Females of cyst nematodes (CNs, *Heterodera* spp. or *Globodera* spp.) at the final stage of their development transform themselves into dead cysts with infective juveniles encapsulated in eggs inside of the cyst. In the genera *Globodera*, *Heterodera*, and *Meloidogyne*, the chromosomal sex determination is absent and the sex ratio may be environmentally influenced (Perry, Moens, 2011). Most cyst nematodes are amphimictic, i. e., their populations consist of both males and females which reproduce sexually. Additional species are classified by Triantaphyllou and Hirschmann (1980) into groups apparently representing three independent lines of evolution toward parthenogenesis. In two of these lines, involving the *H. trifolii* species complex (*H. galeopsidis*, *H. betae*, and others) and *H. sacchari*, parthenogenesis is of the mitotic type. In the third line, represented by *B. betulae*, parthenogenesis is meiotic, this species being cytologically distinct from all others in the presence of the haploid chromosome number. In the root-knot nematodes (*Meloidogyne* spp.), adult males are rare and not required for reproduction. Root-knot nematodes exhibit a range of reproductive modes, including sexuality (amphimixis), facultative sexuality, meiotic parthenogenesis (automixis), and mitotic parthenogenesis (apomixis). Several of the most widespread and economically important species of *Meloidogyne* (*M. incognita*, *M. arenaria*, *M. javanica*) are obligate mitotic parthenogens.

According to their ontogenesis and morphogenesis, the sedentary nematodes are evolutionary advanced parasites. Both the CSs and RKNs have complex interactions with their hosts; their cycles, however, are different. Adaptations of sedentary nematodes to parasitic mode of life are so perfect, that their pest control is a very complicated task. The progress in understanding of nematode-plant interrelations affords evaluation of incomparable mode of mutual adaptations and dynamic equilibrium between the HPS partners (Perry, Moens, 2011).

Sedentary nematodes have developed adaptive strategies associated with transformations of normal host cells into metabolic active ones that provide nematode food requirements and do not affect plant metabolism in general. Infective parasite juveniles are responsible for the formation of modified host cells. Relationships between the sedentary nematode and the plant host develop in accordance with the parasite life cycle. After the first molting within the egg shell, the invasive second stage juvenile (J2) possessing well developed stylet, hatches from the egg shell. Only J2 of sedentary nematodes can infect plant roots. They penetrate the root tip just in front of the root cap, piercing epidermal cell surface with the stylet and releasing pharyngeal glands enzymes from the stylet tip. These enzymes cause cell wall degradation. After entering the root, larvae move towards the central cylinder (the stele). Juveniles of RKNs and CNs migrate differently: whereas RKN J2 moves intercellularly, the cyst nematode J2 moves intracellularly causing much more damage to host tissues. Upon arrival in the final localization site, larvae position themselves in parallel to the central cylinder (the stele), become motionless, and develop the nematode feeding site, NFS (Gheysen, Fenoll, 2002, Gheysen, Mitchum, 2008).

Irrespective of the nematode-plant combination, the mechanism of NFS induction is similar in the CNs and RKNs. Secretory discharge of nematodes induces for-

mation of special structures that ensure parasite feeding. In the RKN parasitism these are several giant cells, the structure of which develops during hypertrophy and anomalies of cell division. After J2 invasion, abnormal division of the NFS initial nurse cell results in numerous cytokinesis-free nucleic mitoses. The general characteristics of the giant cells are the following: they possess lobed and swollen nuclei that can be very numerous (up to 100); the nucleoli are enlarged; the cytoplasm is grained and electron dense; the vacuoles are small or missing; the walls are irregularly thickened with ingrowths inside the cell; the number of mitochondria and plastids highly increase. The cytoplasm is dense and grainy; it contains lipids, nucleic acids, and proteins, with protein content 10 times as much as the normal content. Cell membranes of giant cells were found to contain cellulose and pectin, but they contain no lignin and suberin; the cells contain no starch but numerous vacuoles. Plant cells, that surround the NFS, divide and swell causing root-knot formation. The emergence and development of root-knots are closely related to the life cycle of RKNs.

Cyst nematodes (CNs) produce a syncytium which can include up to 200 cells. The cells surrounding the nematode are necrotized. The presence of local necroses near feeding sites is a characteristic sign of CNs location in the host root. Local necroses are results of mechanical cells' damage caused by larvae motion through plant tissues. Parenchymal root cells adjacent to the parasite localization are hypertrophied. They are larger than normal parenchymal cells. Alongside with the hypertrophy of the crust cells, their proliferation is observed. The pericycle cells also proliferate. Thus a syncytium is formed.

The main purpose of induction of both RKN giant cells and CN syncytium is to provide the optimum conditions for nematode feeding and development. Duration of development of post-invaded J2 coincides with the duration of the NFS initiation. There are some assumptions about the J2-secreted signal molecules in the development of giant cells and syncytia. Nematode-induced nurse structures in plant tissues are analyzed in detail in several reviews (Gheysen, Fenoll, 2002; Gheysen, Mitchum, 2011; Vieira et al., 2012a, b; Kyndt et al., 2013).

Upon the formation of the feeding site (giant cells or the syncytium), larvae molt thrice. Development of both RKNs and CNs includes a series of morphological changes: they grow in size and change the body shape: being initially vermiform, later they become lemon-shaped or pear-shaped. The intestine is reduced, and ovaries with eggs are formed and enlarged within the body cavity. During feeding of the sedentary endoparasitic nematode, its pharyngeal DG secretion is injected through the stylet into the host feeding cell cytoplasm via «the feeding tube». This tube is formed within the plant cell from the compounds secreted by DG, and acts as a particle filter to stop large ingested molecules that can clog the stylet hole. The crystalline feeding tube is the unique HPR adaptation unknown in other parasitic taxa. Study of these formations using fluorescent inclusions showed that they let pass 20—40 kDa particles. The plasmalemma remains intact at initial stages of nematode feeding. The nematode takes nutrients from the cytosol through small pores in the plasmalemmas produced in the place of their contact with the stylet hole. In the site where the edge of the stylet is immersed into the host modified cell, a plug formed of protein products secreted by amphids grows, and the parasite entry site becomes «sealed up». Investigation of the feeding processes of the cyst nematode *Heterodera schachtii* showed that the secretory proteins released by amphids in intercellular space

between the nematode head and cell wall fix (glue) the nematode head to the syncytium wall (Hussey, Mims, 1991; Hussey, Grundler, 1998; Davis et al., 2004).

About one week after the final fourth molt, RKN female starts secreting gelatinous matrix sac where it lays eggs. Eggs in the gelatinous matrix, which consists primarily of carbohydrate, may be partially protected by the presence of antimicrobial compounds such as chitinase and polyphenoloxidase.

Reproduction of CNs always requires males; after fertilization, the eggs remain inside the female, and their number increases with age. They gradually fill the entire body of the female. After death, the female turns into a cyst, a bag with eggs (Gheysen, Mitchum, 2011). In some species, when conditions are not favorable for plant growth and infection, eggs within the cyst are especially persistent. Under these conditions they can persist in soil for many years. The incidence of the diapause varies greatly among species and between populations of the same species from different climatic areas. In the absence of a host plant and biological antagonists, the combination of the diapause and quiescence enables *Globodera rostochiensis* to survive in soil for more than 20 years (Wright, Perry, 2006).

Nematode invasion of roots and migration to their feeding sites results in root structure changes and significant reductions in nutrient and water uptake and consequent crop yields. The host-parasite relationship is governed by a complex network of interactions and in susceptible interactions there is a subtle interplay between parasite survival strategies and host defense mechanisms (Curtis, 2007a; Curtis, 2008).

NEMATODE ADAPTATIONS TO PARASITISM

Sedentary endoparasites are able to manipulate plant cell biology by injecting pharyngeal secretions. A great progress in analysing the nature and function of plant parasitic nematodes secretions, nowadays often referred to as effectors (Haegeman et al., 2012; Hewezi, Baum, 2013), was reached in 2000s years. Hogenhout et al. (2009) define effectors as «all pathogen/pest proteins and small molecules that alter host-cell structure and function». Nematode effectors consist of proteins and other molecules secreted by parasites to facilitate penetration and migration in the plant root, to prevent or counteract plant defense response, and to initiate or maintain the development of NFS (Gheysen, Mitchum, 2011; Haegeman et al., 2012; Rosso et al., 2012; Hewezi, Baum, 2013; Mitchum et al., 2013). Effectors from the nematode are the primary signals at the host interface; they are considered to contain nematode pathogenicity factors (Davis et al., 2000, Davis et al., 2004; Vanholme et al., 2004; Chen et al., 2005; Baum et al., 2007; Davis et al., 2008; Patel et al., 2010; Hewezi, Baum, 2013).

Particular attention in the research of phytonematode effector proteins was focused on cloning and characterization of nematode parasitism genes that encoded parasitism effector proteins produced by the pharyngeal glands (dorsal, DG and subventral, SVGs) and ejected through the nematode's stylet. In addition, putative effector proteins may be produced in the amphids, two chemosensory organs on the head of the nematode; cuticle surface coat may also contain these proteins. The genes of effector proteins («parasitomes») are called «parasitism genes» (Davis et al., 2000, 2004; Greenbaum et al., 2001). To date, over 100 parasitism genes have been cloned, and the host targets and functions of se-

Some effectors secreted by plant-parasitic nematodes

Compound	Nematode species	Organisms with homologous products	Possible function	References
1—4 endonucleases (cellulases)	*Globodera rostochiensis*, *G. pallida*, *G. tabacum*, *Heterodera glycines*, *H. schachtii*, *Meloidogyne incognita*	Bacteria	Cell wall degradation (CWD)	Goellner et al., 2000; Smant et al., 1998; de Muetter et al., 2001; Rosso et al, 1999; Yan et al., 2001; Gao et al., 2002
Pectate lyase	*M. javanica*, *M. incognita*, *G. rostochiensis*, *H. glycines*	Bacteria and fungi	CWD	Doyle, Lambert, 2002; De Boer et al., 2002; Popeijus et al., 2000
Polygalacturonase	*M. incognita*	Bacteria	CWD	Jaubert et al., 2002a
Chorismate mutase	*H. glycines*, *M. javanica*, *M. incognita*, *G. rostochiensis*	Bacteria	Auxin balance regulation, formation of feeding cells, hormone and/or defense	Doyle, Lambert, 2003; Bekal et al., 2003; Popeijus et al., 2000
Thyroxidin peroxidase	*G. rostochiensis*	Animal parasite nematodes	Peroxide destruction, protection against host	Robertson et al., 2000
Allergen — like proteins	*M. incognita*, *H. glycines*	*C. elegans*	Early parasitism?	Ding et al., 2000; Gao et al., 2001
Calreticulin	*M. incognita*	Animal parasite nematodes	Regulate a wide variety of physiological and developmental processes	Jaubert et al., 2002b; Jaoannet et al., 2013
Chitinase	*H. glycines*	*C. elegans*	Unknown	Gao et al., 2002
(CLE)-like proteins	*Globodera* spp., *Heterodera* spp., *Meloidogyne* spp.	Plants	CLEs is involved in promoting the differentiation of stems cells in shoot and root meristems	Guo et al., 2011; Huang et al., 2006
Cellulose binding protein (CBP)	*M. incognita*, *H. schachtii*, *H. glycines*	Plants, Bacteria, Fungus	Cell wall modification	Ding et al., 1998; Gao et al., 2003; Hewezi et al., 2008
Expansin	*G. rostochiensis*	Plants	Induced plant hormones	Qin et al., 2004
Annexins	*H. glycines*, *H. schachtii*	Plants, Animals	Suppression of basal defense responses to promote compatibility	Gao et al., 2003; Fioretti et al., 2002
Fatty acid and retinol binding protein	*Globodera pallida*	Plants, Animals	Protection against plant defense response	Prior et al., 2001
Glutathione peroxidase	*Globodera rostochiensis*	Plants, Animals	Protection against plant defense response	Jones et al., 2004

SPRYSEC	»	»		NB-LRR-resistant protein potato GPA-2	Sacco et al., 2009
Peroxiredoxin	»	»	Plants, Animals	Protection against plant defense response	Robertson et al., 2000
Ubiquitin extension protein	*Heterodera* sp.		*C. elegans*, Plants	Synthesis	Tytgat et al., 2004
Nuclear localisation signals (NLSs)	*Globodera* spp.	*Heterodera* spp. *Meloidogyne* spp.	Proteins of viruses, bacteria, animals, plants and fungi	Unknown	Gao et al., 2003; Elling et al., 2007; Huang et al., 2003; Roze et al., 2008; Jaouannet et al., 2012

veral of the secreted effector proteins have been elucidated. Chemicals secreted by nematodes are listed in the table.

The SVGs are the key producers of plant cell wall modifying proteins that facilitate migration of the nematode through plant tissues. The secreted protein mixture consists of a variety of enzymes that degrade cellulose, hemi-cellulose or pectin and proteins that bind cell wall components to enable or accelerate the digestion process (e. g. cellulose binding proteins) and expansins (Hussy, 1989; Davis et al., 2000; Gao et al., 2002; Gao et al., 2003; Zinovieva et al., 2004; Qin et al., 2004, Hewezi et al., 2008). The cell wall degrading enzymes (CWDEs) differ from those observed in other animals. According to the gene databases, CWDEs are presumably acquired through horizontal gene transfer (HGT) from bacteria (Smant et al., 1998; Yan et al., 1998; Scholl et al., 2003; Jones et al., 2005; Mitreva et al., 2009; Danchin et al., 2010).

The strategy of sedentary PPNs appears to involve the alteration of endogenous plant cell mechanisms accomplished by suite of effector proteins. The studies have provided a new insight onto potential functions of a number of effectors, enabling nematode's ability to modulate plant stress and defense responses to establish HPS compatibility. Various effectors that affect plant stress and defense responses have been actually characterized (Smant, Jones, 2011; Quentin, 2013). Annexins are calcium and phospholipid binding proteins involved in a variety of cellular and physiological processes associated with abiotic stress responses in plants (Jaubert et al., 2002a, b). Hs10A06 effector targets *Arabidopsis* spermidine synthase 2. Plants overproducing Hs10A06 are more susceptible to CNs and to bacterial and viral pathogens and produce smaller amounts of pathogenesis-related (PR) proteins. Hs10A06 acts on salicylic acid signaling and on the antioxidant machinery, thereby protecting nematodes against plant defense responses (Hewezi et al., 2010). Similarly, the Hs4F01 annexin-like effector is secreted into the cytosol (Patel et al., 2010), where it interacts with an oxidoreductase of the 2OG-Fe(II) oxygenase family to prevent the triggering of host defense. Another CN effector, Hg30C02, interacts physically with a plant

â-1,3-endoglucanase, a potential PR protein, and may thus be involved in defense suppression (Hamamouch et al., 2012).

An intriguing parasitic tactics employed by nematodes to accomplish this reprogramming is the molecular mimicry of host proteins. Nematode secreted ubiquitin extension proteins may alter cellular proteins degradation pathways or act as signaling molecules (Tytgat et al., 2004).

Nematodes have been shown to mimic endogenous host plant proteins by secreting chorismate mutase, expansin-like proteins, CLAVATA3/ESR (CLE)-like proteins, and annexins (Curtis et al., 2011; Replogle et al., 2011). Identification of functional expansin-like enzymes (that break non-covalent linkages between cellulose chains) and CLE-like proteins impart nematodes with the unique ability to manipulate host developmental programs directly (reviewed in Curtis, 2007a). CLEs are a class of plant peptide hormones that regulate a wide variety of physiological and developmental processes. For example, a subset of CLEs is involved in promoting the differentiation of stem cells in shoot and root meristems (Curtis, 2007b). The remarkable sequence and functional similarity between plant and cyst nematode CLEs suggests a potential role of nematode CLE mimics in repressing cell proliferation to promote differentiation. This is consistent with the observation that the syncytium development resembles the xylem differentiation.

Gene 16D10 was also isolated from subventral pharyngeal glands (SVGs) of *Meloidogyne incognita*. The secretory peptide of 16D10 gene significantly stimulates host root proliferation with normal differentiation. This bioactive RKN peptide directly interacts with plant SCARECROW-like (SCL) transcription factors that, presumably, have important roles in plant growth and development (Huang et al., 2006).

Furthermore, both CNs and RKNs secretory proteins are homologous to plant chorismate mutases (Bekal et al., 2003; Jones et al., 2003; Huang et al., 2005; Vanholme et al., 2009). The nematode chorismate mutases affect the plant shikimate pathway, thereby decreasing the synthesis of salicylic acid and phytoalexin through competition with chorismate and preventing triggering of the host defense. The overexpression of nematode chorismate mutases *in planta* alters root; it has been suggested that these effectors affect the auxin pool within host cells (Doyle, Lambert, 2003; Huang et al., 2005).

The recently characterized *M. incognita* effector Mi8D05 affects different function of plant cells (Xue et al., 2013). This effector has been shown to interact with a plant tonoplast aquaporin intrinsic protein (TIP2), suggesting a role in the regulation of solute and water transport within giant cells, thus promoting giant cell enlargement and nematode feeding (Quentin et al., 2013).

It remains unclear, how the secretion of specific effectors triggers the infection process in different host tissues and cells. Some effectors (e. g. endoglucanases (ENGs)) are distinctly secreted into the apoplast during nematode intra- or intercellular migration in the root (Rosso et al.,1999, 2011; Vieira et al., 2011), whereas other effectors interact directly with specific cytoplasmic proteins (Hewezi, Baum, 2013). Recent studies have shown that some effectors contain nuclear localization signals (NLSs) that mediate protein targeting to the nucleus (Elling et al., 2007; Tytgat et al., 2004). Bioinformatics analyses of predicted effectors have revealed the presence of NLSs in several secreted proteins from both CNs (Gao et al., 2003; Elling et al., 2007) and RKNs (Huang et al., 2003; Roze et al., 2008). Immunostainings demonstrated that MiEFF1 is produced in

the dorsal pharyngeal gland of the nematode and is ejected through the stylet into the giant cells where it is transported into nuclei (Jaouannet et al., 2012). Hewezi and Baum (2013) have suggested that the CN effectors recruit proteins involved in nucleocytoplasmic transport and nuclear dynamics during the infection. The manipulation of host cell processes, such as the cell cycle, gene expression, and immunity, involves the targeting of the host nucleus by the nematode secreted effectors (Quentin, et al., 2013).

The cuticle surface coat (SC) of the J2 of plant-parasitic nematodes is considered to be involved in interactions with the host plant. Materials present in the nematode SC mask the nematode surface, thus playing a role in suppression or modulation of the host defense response. In *G. pallida*, Gp-FAR-1, a secreted lipid binding protein of the J2 SC binds a wide range of fatty acids including linoleic and linolenic acids (Prior et al., 2001). These fatty acids are metabolized by lipoxygenase as a part of the signaling pathway leading to the production of jasmonic acid which inhibits nematode development *in vitro* (Zinovieva et al., 2013). A range of enzymes that may neutralize active oxygen species produced by the host have been described from the nematode surface. A peroxiredoxin (thioredoxin peroxidase) secreted in great quantities by the surface of the potato cyst nematode *G. rostochiensis* was found to catalyze the breakdown of hydrogen peroxide but, unlike most proteins of this type, did not metabolize larger lipid hydroperoxides (Robertson et al., 2000). Genome sequencing projects for *M. incognita* and *M. hapla* (Abad et al., 2008; Opperman et al., 2008) demonstrated that similar proteins were also present in RKNs (Curtis et al., 2011).

Phytohormones such as auxin and cytokinin, as well as other molecules, are present in root diffusates; they act as the signal molecules to prepare J2 of *Meloidogyne* sp. for root infection by inducing changes in the nematode SC and nematode behavior which is essential for the host infection (Wubben et al., 2001; Ithal et. al., 2007). This significant increase in the lipophilicity of the nematode SC might play an important role in altering the permeability barrier of the nematode cuticle and, therefore, control the uptake of water, ions and lipids in the nematode, which are important in the cell signaling pathways (Geldner, 2001).

Two bilaterally symmetrical amphids in the nematode head are the main nematode chemosensory organs involved in host-recognition processes. Nematodes rely on chemoreception to find a host in soil. When a root is encountered, its surface is explored for a suitable penetration site. Chemoreception might also be involved in helping nematodes define the suitable root tip cells to initiate their NFS. The secretions released from nematode amphids participate in the formation of the feeding plug, sealing an orifice of the nematode stylet insertion into the root cell. A putative avirulence gene of the RKN encodes the secreted amphidial protein (Smant et al., 1998).

Three categories of plant genes are involved in host resistance response to nematode invasion: (1) genes inducing plant defense; (2) stress-related genes, and (3) genes involved in nematode feeding, including formations of syncytium or giant cells. Most of these genes are induced in both compatible and incompatible HPRs, albeit with differences in their expression levels and timing. Parasitism proteins in nematode-plant interactions may function as extracellular or intracellular ligands or signal transduction components; they may be transported into the nucleus, or they may affect cytoplasmic components; all the actions could modify the recipient plant cell.

HOST'S MOLECULAR RESPONSES

Targets of nematode effectors suggest their differential roles in host cell transcriptional, developmental, and metabolic reprogramming, protein degradation, and phytohormone transport and accumulation (Fuller et al., 2008; Gheysen, Mitchum, 2011; Haegeman et al., 2012; Hewezi and Baum, 2013). Some nematode molecules may be recognized by receptors in host cells and can play the role of initial signals to trigger plant resistance response. Plants have devised sophisticated and multi-faceted defense mechanisms. There are, in essence, two branches of the plant immune system. The older one, the basic immunity, is triggered by pathogen-associated molecular patterns (PAMP- or MAMP triggered immunity, PTI); the second one, the effector-triggered immunity (ETI), relies on resistance (R) proteins.

A comprehensive view of the multifaceted interactions between plants and pathogens is illustrated by the «*zigzag*» model assumed by Jones and Dangl (2006). This model proposes that the first line of active plant defense is formed by pattern recognition receptors (PRRs). These are cell surface receptors that recognize PAMPs and initiate an array of basal defense responses, PTI. To date little is known about nematode molecules acting as PAMPs in plants. A recent paper reported a PAMP triggered immune response to derivatives of chitins, suggesting that cuticular substances of PPNs and secreted proteins and products of cell wall degradation may act as PAMPs. Transcriptomic analysis has shown that a massive down-regulation of genes involved in plant defense is associated with the early stages of plant—nematode interaction (Jammes et al., 2005; Barcala et al., 2010; Damiani et al., 2012).

Successful pathogens are able to overcome PTI by means of secreted effectors that suppress PTI responses, resulting in effector-triggered susceptibility. During evolution, plants have responded to these effectors through the development of cytoplasmic R proteins that recognize the presence or activity of single effectors.

The R-gene products can be categorized into two main classes based on conserved structural features (Dangl, Jones, 2001; Chisholm et al., 2006). The largest class of R proteins (called the NBS-LRR class of R proteins) possesses the central nucleotide-binding site (NBS) domain in addition to a leucine-rich repeat (LRR). The second major class of R-genes encodes extracellular LRR (eLRR) proteins. These R proteins activate effector-triggered immunity (ETI). Several major resistance genes encoding ETI receptors with recognition specificity to populations of biotrophic nematodes have been found in a wide range of crops and only eight nematode R-genes have been cloned and demonstrated to confer resistance to RKNs or CNs (Kaloshian et al., 2011).

As a result of the selection pressure, the pathogens evolved that have either lost or altered the effector recognized by the host or that have gained novel effectors to suppress the ETI response. In its turn, new plant receptors evolved that either recognized the obvious effectors or the newly acquired effectors, resulting again in ETI. This coevolution proceeds, with continuous selection for novel pathogen isolates that overcome ETI and new plant genotypes that resurrect ETI.

In accordance with the «*zigzag*» model, plant pathologists discriminate two phases of plant immunity: PTI triggered by PAMPs and ETI triggered by effectors, with the paradigm that activated immune responses in ETI occur quicker and are more prolonged and more robust than those in PTI (Jones, Dangl, 2006).

The avirulence gene product is expected to be co-localized with the R-gene product. The structure of Gpa2 and Mi-1 suggests that these proteins are localized in the cytoplasm of the plant cell. Hence, nematode secretions that are injected into the nurse cell might interact with this class of R-genes. In case of Rhg1, Rhg4, and Hs1pro1, the LRR domain is predicted to be localized in the extracellular space. Therefore, the putative Avr-products from nematodes most probably originate from the nematode SC, the amphids, or the pharyngeal SVGs.

Plants have evolved resistance proteins that can recognize, either directly or indirectly, pathogen effectors, and induce effector-triggered immunity (ETI) (Jones, Dangl, 2006). However, very few nematode avirulence effectors have been identified (Smant, Jones, 2011). Recently, the genomes of two near isogenic virulent and avirulent lines of *Meloidogyne incognita* were analyzed using AFLP. One of the polymorphic fragments present in the virulent line and absent in the avirulent line, map-1 encodes a secretory protein that is produced by the amphids (Semblat et al., 2001). A more complete example of a nematode effector linked to ETI is the Gp-RBP-1 SPRYSEC gene from *Globodera pallida* (Sacco et al., 2009). Transient co-expression of Gp-RBP-1 and the nematode resistance gene Gpa-2, which encodes a CC-NB-LRR type immune receptor, in leaf tissues induces a specific hypersensitive response (HR), making this effector the likely cause of avirulence in nematode populations. Interestingly, Gp-RBP-1 variants capable of inducing a Gpa-2 dependent HR have been found in virulent nematode populations, suggesting that these virulent nematodes have evolved other effectors that suppress Gpa-2 mediated ETI.

However, accumulating evidence indicates that the separation between PAMPs and effectors, and between PRRs and R-proteins, and thus also between PTI and ETI, cannot be maintained strictly. Rather, there is a continuum between PTI and ETI (Hewezi, Baum, 2013).

If an HR is induced in cells adjacent to the migratory track, it is likely that the invading nematode is recognized at an early stage of the infection process. If, however, the nurse cell development is arrested in a resistant plant, recognition of the nematode could occur at a later stage. During invasion, close contact occurs between plant cells and the nematode SC. In addition, secretions are released by the amphids, sense organs involved in chemotaxis. Salivary proteins are secreted both for enzymatic degradation of cell walls during migration and for the induction of the nurse cell.

In resistant plants, the feeding cell initiation and development are arrested, resulting in the nematode starvation. Two major types of resistance responses can be distinguished based on extensive microscopic observations of several incompatible plant-nematode interactions (Tomczak et al., 2009). The first type is associated with programmed cell death, a response which is referred to as the hypersensitive response (HR), and systemic acquired resistance (SAR) in the host. The HR is accompanied by an oxidative burst resulting in the production of hydrogen peroxide H_2O_2 (Waetzig et al., 1999) and the accumulation of phenylpropanoid compounds (Robinson et al., 1988).

The second type blocks the development of the host nurse cell at the late infection stage. By contrast, the late resistance response is characterized by the degeneration of the NFS around two weeks post infection and the absence of HR. This resistance mechanism has been observed in a wide range of incompatible plant—nematode interactions. Initially, no distinct differences can be ob-

served between the compatible and the incompatible interaction. The nematode is able to establish a functional NFS enabling the development of males and females. In later stages, however, differences in morphology of feeding cells are observed. In resistant plants, the proliferation of the nurse cell is arrested resulting in less dense cytoplasm and more vacuoles compared to nurse cells induced in a susceptible plant. Moreover, it is often observed that the connection between the nurse cell and the vascular tissue is less pronounced. These features indicate that the metabolic activity of the nurse cell is reduced as a result of the resistance response. Finally, cells adjacent to the NFS become necrotic; this necrosis is followed by the degradation of the nurse cell itself. These resistance mechanisms result in the limitation of nutrients at the late stage of nematode development, after sex determination. In the case of the slow defense response, nematode females in plants are relatively numerous. However, food supply is insufficient, resulting in the arrestment of female development and reproduction. Defense responses include the production of toxic oxygen radicals and systemic signaling compounds as well as the activation of defense genes that lead to the production of structural barriers or other toxins (Robinson et al., 1988).

Genes encoding direct defense proteins that are activated, include peroxidase, chitinase, lipoxygenase, extensin and proteinase inhibitors. Genes that encode enzymes in pathways that result in the synthesis of other defense compounds are also activated during plant defense responses. For example, genes encoding enzymes that lead to the synthesis of phytoalexins or deposition of physical barriers such as callose and lignin are induced at the early phases of the nematode infection process (Gheysen, Jones, 2006).

BIOCHEMICAL FACTORS OF PLANT RESISTANCE TO PARASITIC NEMATODES

Chemicals involved in plant responses to pest infections are classified into phytoanticipins and phytoalexins. The first are low-molecular anti-microbial substances being present in a plant prior to infection, or produced from earlier precursors; the latter are low-molecular anti-microbial substances being synthesized and accumulated in plants *de novo* (Zinovieva et al., 2004).

Phytoanticipins, represented by phenols, terpenoids, glycosides and several other compounds of plant specialized metabolism are related to constitutional compounds in plant tissues which are considered to be responsible for plant resistance to nematodes. In infested plants, the production of these compounds increases, especially in the resistant varieties.

Phytoalexins (PhA) are low-molecular anti-microbial compounds synthesized and accumulated in plants *de novo*. PhA have been found and described hitherto only in plant species belonging to families Fabaceae, Malvaceae, Solanaceae, and Musaceae. For a long time PhA were considered as the main compartments of plant resistance mechanisms. Later, however, aside from PhA, other active protective proteins, proteinase inhibitors, and active oxygen species, acting in other types of immune responses, have been revealed.

PR proteins (pathogenesis-related proteins) formation is one of the main plant protective reactions. In healthy plants, PR-proteins are present only in small amounts.

Proteinase inhibitors (PI) fulfill a number of functions in plants serving as a part of the protective system. The inhibition of proteolytic reactions may lead to violation of parasite ability to digest plant proteins and, consequently, inhibit the nematode growth and development.

Food factors. Plant resistance may be associated with availability of compounds which are necessary for PPNs but not synthesized in them. The amino acids: lysine, leucine, methionine, phenylalanine, histidine and tryptophane, are especially indispensable.

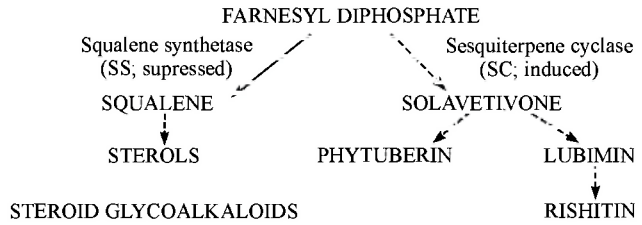
The plant defense response leads to elimination of substances necessary for parasites. Sterols are of particular importance. Nematodes are unable to synthesize them and use the plant hosts' sterols. As response to the infection in the system «tomatoes — RKN *Meloidogyne incognita*», the sterols' level increases after infection only in susceptible varieties, and decreases in the resistant ones (Zinovieva et al., 1990).

CONCLUSIONS

PPNs possess structural adaptations to parasitic mode of life in plant tissues. These adaptations include formation of the stylet for penetration of plant cell walls, and pharyngeal glands (dorsal and subventral) and amphids, which release secretions into plant tissues. Secretions of pharyngeal glands play the key role in HPRs. Substances injected by nematodes may, in many cases, bind to receptors of plant cells and affect (via plant signaling systems) the expression of genes that determine plant response, thereby acting as inducers or elicitors of defense reactions. Elicitors act as signals that activate the intricate chain of processes inducing and regulating plant responses. In resistant plants, the nurse cell initiation and its development are arrested, resulting in starvation of the nematode. Defense responses include the production of toxic oxygen radicals and systemic signaling compounds as well as the activation of defense genes that lead to the production of structural barriers or other toxins. Adaptive responses of plants to nematode infections vary depending on the level of nematode adaptation to parasitism. In research on relationships of migratory and sedentary nematodes, parasitizing certain Solanaceae plants (potatoes and tomatoes) it has been shown, that the plant host is capable to react adequately to the specific parasite influence (Zinovieva, 1986). The migratory parasites move through root tissues, causing extensive cellular necrosis; plant responses are expressed in producing of substances (phenols and steroid glycosides) limiting nematode dissemination inside host tissues.

Completely different strategy of plant resistance is observed when plant hosts are affected by the sedentary nematodes, the most evolutionary advanced group of PPNs. They have developed adaptive strategies associated with the transformation of normal plant root cells into metabolically active ones, which ensure nematode feeding requirements. These immobile gall-dwelling nematodes significantly affect plants chemically and consuming nutrients. The plant defense mechanisms are expressed not only in the protein inhibition but also in the formation of the «hungry area» surrounding the NFS.

PPNs do not synthesize sterols which they need for development and pathogenicity. The plant response is associated with the decrease in quantitative and



Regulation of isoprenoid biosynthesis in tomato root and resistance plants.

Normal sterol and steroid glycoalkaloids synthesis pathways are suppressed in favor of sesquiterpenoid phytoalexin synthesis during the plant host hypersensitive response.

qualitative sterol contents (Zinovieva et al.,1990). Reduction of sterols in plant roots is associated with accumulation of sesquiterpene phytoalexins, rishitine, and lubimine, that have a common biosynthetic pathway with sterols (see figure). It is suggested that terpene biogenesis has switched from a healthy plant sterol formation pathway, to the formation of highly toxic phytoalexins. As a result, the parasite loses necessary nutrients for its development and simultaneously the nematode is affected by plant phytoalexins. Changes in the biosynthesis pathway, leading to the formation of the new molecule type, are regarded as the most complex biochemical adaptations in terms of evolution (Hochachka, Somero, 2002).

Mutual adaptations (co-adaptations) are revealed in the «nematode — plant» system. The parasite species has developed its morphological and biological adaptations to infect the host and induce the NFS, whereas the host develops its defense responses at the organism, tissue and cell levels. Relationships in the HPS have various finals. The strong host resistance response leads to the death of the parasite. If the parasite possesses the high biochemical pathogenic adaptations and protective mechanisms of the plant are insufficient, the disease develops which can lead to plant host death. If the HPRs are rather balanced, it leads to smoothing of the antagonistic relations and the formation of the equilibrium HPS.

References

Abad P., Gouzy J., Aury J. M., Castagnone-Sereno P., Danchin E. G., Deleury E., Perfus-Barbeoch L., Anthouard V., Artiguenave F., Blok V. C., Caillaud M. C., Coutinho P. M., Dasilva C., De Luca F., Deau F., Esquibet M., Flutre T., Goldstone J. V., Hamamouch N., Hewezi T., Jaillon O., Jubin C., Leonetti P., Magliano M., Maier T. R., Markov G. V., McVeigh P., Pesole G., Poulain J., Robinson-Rechavi M., Sallet E., Ségurens B., Steinbach D., Tytgat T., Ugarte E., van Ghelder C., Veronico P., Baum T. J., Blaxter M., Bleve-Zacheo T., Davis E. L., Ewbank J. J., Favery B., Grenier E., Henrissat B., Jones J. T., Laudet V., Maule A. G., Quesneville H., Rosso M. N., Schiex T., Smant G., Weissenbach J., Wincker P. 2008. Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. Nature Biotechnology. 26 (8) : 909—915.

Anderson R. V., Byer J. R. 1975. Ultrastructure of the esophageal procorpus in the plant parasitic nematode, *Tylenchorhynchus dubius*, and functional aspects in relation to feeding. Canadian Journal of Zoology. 53 (11) : 1581—1595.

Barcala M., Garcia A., Cabrera J., Casson S., Lindsey K., Favery B., Garcia-Casado G., Solano R., Fenoll C., Escobar C. 2010. Early transcriptomic events in microdissected *Arabidopsis* nematode-induced giant cells. The Plant Journal. 61 (4) : 698—712.

Baum T. J., Hussey R. S., Davis E. L. 2007. Root-knot and cyst nematode parasitism gene the molecular basis of plant parasitism. In: Genetic Engineering, Principles and Methods, Ed. J. K. Setlow. Springer Science +Business Media, LLC. 28 : 17—43.

Bekal S., Niblack T. L., Lambert K. N. 2003. A chorismate mutase from the soybean cyst nematode *Heterodera glycines* show spolymorphisms that correlate withn virulence. Molecular Plant—Microbe Interactions. 16 (5) : 439—446.

Bird D. M., Kaloshian I. 2003. Are roots special? Nematodes have their say. Physiology and Molecular Plant Pathology. 62 (2) : 115—123.

Brown D. J. F., Robertson W. M., Trudgill D. L. 1995. Transmission of viruses by plant nematodes. Annual Review of Phytopathology. 33 : 223—249.

Chen Q., Rehman S., Smant G., Jones J. T. 2005. Functional analysis of pathogenicity proteins of the potato cyst nematode *Globodera rostochiensis* using RNAi. Molecular Plant—Microbe Interactions. 18 (7) : 621—625.

Chisholm S. T., Coaker G., Day B., Staskawicz B. J. 2006. Host-microbe interactions: shaping the evolution of the plant immune response. Cell. 124 (4) : 803—814.

Chitwood D. J. 2003. Research on plant-parasitic nematode biology conducted by the United States Department of Agriculture — Agricultural Research Service. Pest Management Science. 59 : 748—753.

Combes C. 2005. The art of being a parasite. Chicago, University of Chicago Press. 291 p.

Curtis R. H. C. 2007a. Plant parasitic nematode proteins and the host—parasite interaction. Briefings in Functional Genomics and Proteomics. 6 (1) : 50—58.

Curtis R. H. C. 2007b. Do phytohormones influence nematode invasion and feeding site establishment? Nematology. 9 (2) : 155—160.

Curtis R. H. C. 2008. Plant-nematode interactions: environmental signals detected by the nematode's chemosensory organs control changes in the surface cuticle and behavior. Parasite. 15 (3) : 310—316.

Curtis R. H. C., Jones J. T., Davies K. G., Sharon E., Spiegel Y. 2011. Plant nematode surfaces. In: Biological control of plant-parasitic nematodes: building coherence between microbial ecology and molecular mechanisms, Springer. 115—144.

Danchin E. G. J., Rosso M. N., Vieira P., de Almeida-Engler J., Coutinho P. N., Henrissat B., Abad P. 2010. Multiple lateral gene transfers followed by duplications promoted plant parasitism ability in nematodes. Proceedings of the National Academy of Sciences of USA 107 : 17 651—17 656.

Dangl J. L., Jones J. D. 2001. Plant pathogens and integrated defense responses to infection. Nature. 411 : 826—833.

Damiani I., Baldacci-Cresp F., Hopkins J., Andrio E., Balzergue S., Lecomte P., Puppo A., Abad P., Favery B., Herouart D. 2012. Plant genes involved in harboring symbiotic rhizobia or pathogenic nematodes. New Phytologist. 194 (2) : 511—522.

Davis E. L., Hussey R. S., Baum T. J. 2004. Getting to the roots of parasitism by nematodes.Trends in Parasitology. 20 (3) : 134—141.

Davis E. L., Hussey R. S., Baum T. J., Bakker J., Schots A., Rosso M.-N. 2000. Nematode parasitism genes. Annual Review of Phytopathology. 38 (1) : 365—396.

Davis E. L., Hussey R. S., Mitchum M. G., Baum T. J. 2008. Parasitism proteins in nematode-plant interactions. Current Opinion in Plant Biology. 11 (4) : 360—366.

De Boer J. M., McDermott J. P., Wang X., Maier T., Qui F., Hussey R. S., Davis E. L., Baum T. J. 2002. The use of DNA microarrays for the developmental expression analysis of cDNAs from the oesophageal gland cell region of *Heterodera glycines*. Molecular Plant Pathology. 3(4) : 261—270.

de Muetter J., Vanholme B., Bauw G., Tytgat T., Gheysen G. 2001. Preparation and sequencing of secreted proteins from the pharyngeal glands of the plant parasitic nematode *Heterodera schachtii*. Molecular Plant Pathology. 2 (5) : 297—301.

Ding X., Shields J., Allen R., Hussey R. S. 1998. A secretory cellulose-binding protein cDNA cloned from the root-knot nematode (*Meloidogyne incognita*). Molecular Plant—Microbe Interactions. 11 (10) : 952—959.
Ding X., Shields J., Allen R., Hussey R. S. 2000. Molecular cloning and characterization of a venom allergen AG5-like cDNA from *Meloidogyne incognita*. International Journal for Parasitology. 30 (1) : 77—81.
Doyle E. A., Lambert K. N. 2002. Cloning and characterization of an esophageal-gland-specific pectate lyase from the root-knot nematode *Meloidogyne javanica*. Molecular Plant—Microbe Interactions. 15 (6) : 549—556.
Doyle E. A., Lambert K. N. 2003. *Meloidogyne javanica* chorismate mutase 1 alters plant cell development. Molecular Plant—Microbe Interactions. 16 (1) : 123—131.
Elling A. A., Davis E. L., Hussey R. S., Baum T. J. 2007. Active uptake cyst nematode parasitism proteins in to the plant cell nucleus. International Journal for Parasitology. (11) : 1269—1279.
Endo B. Y. 1984. Ultraslructure of the esophagus of larvae of the soybean cyst nematode, *Heterodera glycines*. Proceedings of the Helminthological Society of Washington. 51 (1) : 1—24.
Fioretti L., Porter A., Haydock P. J., Curtis R. 2002. Monoclonal antibodies reactive with secreted — excreted products from the amphids and the cuticle surface of *Globodera pallida* affect nematode movement and delay invasion of potato roots. International Journal for Parasitology. 32 (14) : 1709—1718.
Fuller V. L., Lilley C. J., Urwin P. E. 2008. Nematode resistance. New Phytologist. 180 (1) : 27—44.
Hamamouch N., Li C., Hewezi T., Baum T. J., Mitchum M. G., Hussey R. S. et al. 2012. The interaction of the novel 30C02 cyst nematode effector protein with a plant beta-1.3-endoglucanase may suppress host defense to promote parasitism. Journal of Experimental Botany. 63 (10) : 3683—3695.
Haegeman A., Mantelin S., Jones J. T., Gheysen G. 2012. Functional roles of effectors of plant-parasitic nematodes. Gene. 92 (1) : 19—31.
Hewezi T., Baum T. J. 2013. Manipulation of plant cells by cyst and root-knot nematode effectors. Molecular Plant—Microbe Interactions. 26 (1) : 9—16.
Hewezi T., Howe P., Maier T. R., Hussey R. S., Mitchum M. G., Davis E. L., Baum T. J. 2008. Cellulose binding protein from the parasitic nematode *Heterodera schachtii* interacts with *Arabidopsis* pectin methylesterase: cooperative cell wall modification during parasitism. Plant Cell. 20 : 3080—3093.
Hewezi T., Howe P. J., Maier T. R., Hussey R. S., Mitchum M. G., Davis E. L. et al. 2010. *Arabidopsis* spermidine synthase is targeted by an effector protein of the cys tnematode *Heterodera schachtii*. Plant Physiology. 152(2) : 968—984.
Hochachka P. W., Somero G. N. 2002. Biochemical Adaptation: Mechanism and Process in Physiological Evolution. Oxford University Press. 466 p.
Hogenhout S. A., Van der Hoorn R. A., Terauchi R., Kamoun S. 2009. Emerging concepts in effector biology of plant-associated organisms. Molecular Plant—Microbe Interactions. 22 (2) : 115—122.
Huang G., Dong R., Allen R., Davis E. L., Baum T. J., Hussey R. S. 2005. Two chorismate mutase genes from the root-knot nematode *Meloidogyne incognita*. Molecular Plant Pathology. 6 (1) : 23—30.
Huang G., Gao B., Maier T., Allen R., Davis E. L., Baum T. J., Hussey R. S. 2003. A profile of putative parasitism genes expressed in the esophageal gland cells of the root-knot nematode *Meloidogye incognita*. Molecular Plant—Microbe Interactions. 16 (5) : 376—381.
Huang G., Dong R., Allen R., Davis E. L., Baum T. J., Hussey R. S. 2006. A root-knot nematode secretory peptide functions as a ligand for a plant transcription factor. Molecular Plant—Microbe Interactions. 19 (5) : 463—470.
Hussey R. S. 1989. Disease-inducing secretions of plant-parasitic nematode. Annual Review of Phytopathology. 27 : 123—141.
Hussey R. S., Mims C. W. 1990. Ultrastructure of esophageal glands and their secretory granules in the root-knot nematode *Meloidogyne incognita*. Protoplasma. 156 : 9—18.

Hussey R. S., Grundler F. M. W. 1998. Nematode parasitism of plants. In: Physiology and biochemistry of free-living and plant-parasitic nematodes, CAB International Press. 213—243.
Hussey R. S., Mims C. W. 1991. Ultrastructure of feeding tubes formed in giant-cells induced in plants by the root-knot nematode *Meloidogyne incognita*. Protoplasma. 162 : 99—107.
Hussey R. S., Davis E. L., Baum T. J. 2002. Secrets in secretions: genes that control nematode parasitism of plants. Brazilian Journal of Plant Physiology. 14 (3) : 183—194.
Gao B., Allen R., Maier T., Davis E. L., Baum T. J., Hussey R. 2001. Molecular characterization and expression of two venom allergen-like protein genes from *Heterodera glycines*. International Journal for Parasitology. 31 (14) : 1617—1625.
Gao B., Allen R., Maier T., Davis E. L., Baum T. J., Hussey R. S. 2002. Identification of a new beta-1.4-endoglucanase gene expressed in the esophageal subventral gland cells of *Heterodera glycines*. Journal of Nematology. 34 (1) : 12—15.
Gao B., Allen R., Maier T., Davis E. L., Baum T. J., Hussey R. S. 2003. The parasitome of the phytonematode *Heterodera glycines*. Molecular Plant—Microbe Interactions. 16 (8) : 720—726.
Geldner N., Friml J., Stierhof Y. D., Jurgens G., Palme K. 2001. Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. Nature. 413 : 425—428.
Gheysen G., Fenoll C. 2002. Gene expression in nematode feeding sites. Annual Review of Phytopathology. 40 : 191—219.
Gheysen G., Jones I. T. 2006. Molecular aspects of plant-nematode interaction. In: Plant nematology. CABI. N.-Y. 234—255.
Gheysen G., Mitchum M. G. 2008. Molecular insights in the susceptible plant response to nematode infection. In: Plant Cell Monographs, Springer-Verlag Berlin Heidelnerg. 45—82.
Gheysen G., Mitchum M. G. 2011. How nematodes manipulate plant development pathways for Infection. Current Opinion in Plant Biology. 14 (4) : 415—421.
Goellner M., Smant G., De Boer J. M., Baum T. J., Davis E. L. 2000. Isolation of beta-1.4 endoglucanase genes from *Globodera tabacum* and their expression during parasitism. Journal of Nematology. 32 (2) : 154—165.
Greenbaum D., Luscombe N. M., Jansen R., Qian J. 2001. Gerstein M. Interrelating different types of genomic data, from proteome to secretome: forming in on function. Genome Research. 11 (11) : 1463—1468.
Guo Y., Ni J., Denver R., Wang X., Clark S. E. 2011. Mechanisms of molecular mimicry of plant CLE peptide ligands by the parasitic nematode *Globodera rostochiensis*. Plant Physiology. 157 (1) : 476—484.
Ithal N., Recknor J., Nettleton D., Maier T., Baum T. J. et al. 2007. Developmental transcript profiling of cyst nematode feeding cells in soybean roots. Molecular Plant—Microbe Interactions. 20 (5) : 510—525.
Jammes F., Lecomte P., de Almeida-Engler J., Bitton F., Martin-Magniette M.-L., Renou J. P., Abad P., Favery B. 2005. Genome-wide expression profiling of the host response to root-knot nematode infectionin *Arabidopsis*. The Plant Journal. 44 (3) : 447—458.
Jaouannet M., Magliano M., Arguel M. J., Gourgues M., Evangelisti E., Abad P., Rosso M. N. 2013. The root-knot nematode calreticulin Mi-CRTisa key effector in plant defense suppression. Molecular Plant—Microbe Interactions. 26 (1) : 97—105.
Jaouannet M., Perfus-Barbeoch L., Deleury E., Magliano M., Engler G., Vieira P. et al. 2012. A root-knot nematode-secreted protein is injected into giant cells and targeted to the nuclei. New Phytologist. 194 (4) : 924—931.
Jaubert S., Laffaire J.-B., Abad P., Rosso M.-N. 2002a. A polygalacturonase of animal origin isolated from the root-knot nematode *Meloidogyne incognita*. FEBS Letters. 522 : 109—112.
Jaubert S., Ledger T. N., Laffaire J. B., Piotte C., Abad P., Rosso M.-N. 2002b. Direct identification of stylet secreted proteins from root-knot nematodes by a proteomic approach. Molecular and Biochemical Parasitology. 121 (2) : 205—211.
Jones J. D. G., Dangl J. L. 2006. The plant immune system. Nature. 444 : 323—329.

Jones J. T., Furlanetto C., Bakker E., Banks B., Blok V., Chen Q. et al. 2003. Characterization of a chorismate mutase from the potato cyst nematode *Globodera pallida*. Molecular Plant Pathology. 4 (1) : 43—50.

Jones J., Furlanetto C., Kikuchi T. 2005. Horizontal gene transfer from bacteria and fungi as a driving force in the evolution of plant parasitism in nematodes. Nematology. 7 (5) : 641—646.

Jones J. T., Reavy B., Smant G., Prior A. E. 2004. Glutathione peroxidase of the potato cyst nematode *Globodera rostochiensis*. Gene. 324 (1) : 47—54.

Kaloshian I., Olivia J., Desmond O. J., Atamian H. S. 2011. Disease resistance-genes and defense responses during incompatible interactions. In: Genomics and molecular genetics of plant-nematode interactions, Springer Science+Business Media B. V. 309—324.

Kyndt T., Viera P., Gheysen G., de Almeida-Engler J. 2013. Nematode feeding site: unique organs in plant roots. Planta. 238 (5) : 807—818.

Lebedev B. I. 1995. Aspects of the biodiversity and evolutionary parasitology. Vladivostok, Nauka. 208 p.

Lightfoot A. D., Meksem K. 2002. Isolated polynucleotides and polypeptides relating to loci underlying resistance to soybean cyst nematode and soybean sudden death syndrome and methods employing same. U. S. P. N 20020144310.

Milligan S. B., Bodeau J., Yaghoobi J., Kaloshian I., Zabel P., Williamson V. M. 1998. The root-knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. Plant Cell. 10 : 1307—1319.

Mitchum M. G., Hussey R. S., Baum T. J., Wang X., Elling A. A., Wubben M., Davis E. L. 2013. Nematode effector proteins: an emerging paradigm of parasitism. New Phytologist. 199 (4): 879—894.

Mitreva M., Smant G., Helder J. 2009. Role of horizontal gene transfer in the evolution of plant parasitism among nematodes. In: Horizontal gene transfer — genomes in flux, New York, Humana Press. 517—535.

Opperman C. H., Bird D. M., Williamson V. M., Rokhsar D. S., Burke M., Cohn J., Croıner J. 2008. Sequence and genetic map of *Meloidogyne hapla*: A compact nematode genome for plant parasitism. Proceedings of National Academy of Sciences USA. 105 : 14 802—14 807.

Paal J., Henselewski H., Muth J., Meksem K., Menendez C. M., Salamini F., Ballvora A., Gebhardt C. 2004. Molecular cloning of the potato Gro1-4 gene conferring resistance to pathotype Ro1 of the root cyst nematode *Globodera rostochiensis*, based on a candidate gene approach. The Plant Journal. 38 (2) : 285—297.

Patel N., Hamamouch N., Li C., Hewezi T., Hussey R. S., Baum T. J., Mitchum M. G., Davis E. L. 2010. A nematode effector protein similar to annexins in host plants. Journal of Experimental Botany. 61 (1) : 235—248.

Perry R. N., Moens N. 2011. Introduction to plant-parasitic nematodes: modes of parasitism. In: Genomics and Molecular Genetics of Plant-Nematode Interactions, Springer. 3—20.

Popeijus H., Overmars H. A., Jones J., Blok V. C., Goverse A. 2000. Degradation of plant cell walls by nematode. Nature. 406 : 36—37.

Prior A., Jones J. T., Blok V. C., Beauchamp J., McDermott L., Cooper A., Kennedy M. W. 2001. A surface-associated retinol- and fatty acid-binding protein (Gp-FAR-1) from the potato cyst nematode *Globodera pallida*: lipid binding activities, structural analysis and expression pattern. Biochemical Journal. 356 : 387—394.

Qin L., Smant G., Stokkermans J., Bakker J., Schots A., Helder J. 1998. Cloning of a trans-spliced glyceraldehyde-3-phosphate-dehydrogenase gene from the potato cyst nematode *Globodera rostochiensis* and expression of its putative promoter region in *Caenorhabditis elegans*. Molecular and Biochemical Parasitology. 96 (1) : 59—67.

Qin L., Kudla U., Roze E. H., Goverse A., Popeijus H., Nieuwland J., Overmars H., Jones J. T., Schots A., Smant G., Bakker J., Helder J. 2004. Plant degradation: a nematode expansin acting on plants. Nature.427 : 30.

Quentin M., Abad P., Favery B. 2013. Plant parasitic nematode effectors target host defense and nuclear functions to establish feeding cells. Frontiers in Plant Science. Plant-Microbe Interaction. 4 (53) : 1—7.
Replogle A., Wang J., Bleckmann A., Hussey R., Baum T., Sawa S., Davis E. L., Wang X., Simon R., Mitchum M. G. 2011. Nematode CLE signaling in *Arabidopsis* requires CLAVATA2 and CORYNE. The Plant Journal. 65 (3) : 430—440.
Robertson L., Robertson W. M., Sobczak M. et al. 2000. Cloning, expression and functional characterization of a peroxiredoxin from the potato cyst nematode *Globodera rostochiensis*. Molecular and Biochemical Parasitology. 111 (1) : 41—49.
Robinson M. P., Atkinson H. J., Perry R. N. 1988. The association and partial characterisation of a fluorescent hypersensitive response of potato roots to the potato cyst nematodes *Globodera rostochiensis* and *G. pallid*. Revue Nématology. 11 (1) : 99—107.
Rosso M.-N., Favery B., Piotte C., Arthaud L., De Boer J. M., Hussey R. S., Bakker J., Baum T. J., Abad P. 1999. Isolation of a cDNA encoding a beta-1.4-endoglucanase in the root-knot nematode *Meloidogyne incognita* and expression analysis during plant parasitism. Molecular Plant—Microbe Interactions. 12 (7) : 585—591.
Rosso M.-N., Hussey R. S., Davis E. L., Smant G., Baum T. J., Abad P., Mitchum M. G. 2012. Nematode effector proteins: targets and functions in plant parasitism. In: Effectors in plant-microbe interactions, effectors in plant-microbe interactions, Wiley-Blackwell. 327—354.
Rosso M. N., Vieira P., Engler J., Castagnone-Sereno P. 2011. Proteins secreted by root-knot nematodes accumulate in the extracellular compartment during root infection. Plant Signaling and Behavior. 6 (8) : 1232—1234.
Roze E., Hanse B., Mlitreva M., Van Holme B., Bakker J., Smant G. 2008. Mining the secretome of the root-knot nematode *Meloidogyne chitwoodi* for candidate parasitism genes. Molecular Plant Pathology. 9 (1) : 1—10.
Sacco M. A., Koropacka K., Grenier E., Jaubert M. J., Blanchard A., Goverse A., Smant G., Moffett P. 2009. The cyst nematode SPRYSEC protein RBP-1 elicits Gpa2- and RanGAP2-dependent plant cell death. PLoS Pathogens 5(8) e1000564 DOI:10.1371 /journal.ppat.1000564.
Scholl E. H., Thorne J. L., McCarter J. P., Bird D. M. 2003. Horizontally transferred genes in plant parasitic nematodes: a high-through put genomic approach. Genome Biology. 4 (6) : R39, doi:10.1186/gb-2003-4-6-r39.
Semblat J. P., Rosso M. N., Hussey R. S., Abad P., Castagnone-Sereno P. 2001. Molecular cloning of a cDNA encoding an amphid-secreted putative avirulence protein from the root-knot nematode *Meloidogyne incognita*. Molecular Plant-Microbe Interactions. 14 (1) : 72—79.
Smant G., Jones J. 2011. Suppression of plant defenses by nematodes. In: Genomics and Molecular Genetics of Plant-Nematode Interactions, Berlin, Springer. 273—286.
Smant G., Stokkermans J., Yan Y. T., de Boer J. M., Baum T. J., Wang X. H., Hussey R. S., Gommers F. J., Henrissat B., Davis E. L. et al. 1998. Endogenous cellulases in animals: isolation of b-1.4-endoglucanase genes from two species of plantparasitic cyst nematodes. Proceedings of the National Academy of Sciences, USA. 95 (9) : 4906—4911.
Sobczak M., Avrova A., Jupowicz J., Phillips M. S., Ernst K., Kumar A. 2005. Characterization of susceptibility and resistance responses to potato cyst nematode (*Globodera* spp.) infection of tomato lines in the absence and presence of the broad-spectrum nematode resistance *Hero* gene. Molecular Plant—Microbe Interactions. 18 (2) : 158—168.
Triantaphyllou A. C., Hirschmann H. 1980. Cytogenetics and morphology in relation to evolution and speciation of plant-parasitic nematodes. Annual Review of Phytopathology. 18 : 333—359.
Tomczak A., Koropacka K., Smant G., Goverse A., Bakker E. 2009. Resistant Plant Responses. In: Cell Biology of Plant Nematode Parasitism, Heidelberg, Springer-Verlag. 3—115.
Tytgat T., Vanholme B., De Meutter J., Claeys M., Couvreur M., Vanhoutte I., Gheysen G., van Criekinge W., Borgonie G., Coomans A., Ghey-

sen G. 2004. A new class of ubiquitin extension proteins secreted by the dorsal pharyngeal gland in plant parasitic cyst nematodes. Molecular Plant—Microbe Interactions. 17 (8) : 846—852.

van der Vossen E. A. G., Rouppe van der Voort J. N. A. M., Kanyuka K., Bendahmane A., Sandbrink H., Baulcombe D. C., Bakker J., Stiekema W. J., Klein-Lankhorst R. M. 2000. Homologues of a single resistance-gene cluster in potato confer resistance to distinct pathogens: A virus and a nematode. The Plant Journal. 24 (5) : 567—576.

Vanholme B., De Meutter J., Tytgat T., van Montagu M., Coomans A., Gheysen G. 2004. Secretions of plant-parasitic nematodes: a molecular update. Gene. 332 (1) : 13—27.

Vanholme B., Kast P., Haegeman A., Jacob J., Grunewald W., Gheysen G. 2009. Structural and functional investigation of a secreted chorismate mutase from the plant-parasitic nematode *Heterodera schachtii* in the context of related enzymes from diverse origins. Molecular Plant Pathology. 10 (2) : 189—200.

Vieira P., Danchin E. G. J., Neveu C., Croza C., Jaubert S., Hussey R. S. et al. 2011. The plant apoplasm is an important recipient compartment for nematode secreted proteins. Journal of Experimental Botany. 62 (3) : 1241—1253.

Vieira P., Engler G., de Almeida Engler J. 2012a. Whole-mount confocal imaging of nuclei in giant feeding cells induced by root-knot nematodes in *Arabidopsis*. New Phytologist. 195 (2) : 488—496.

Vieira P., Banora M. Y., Castagnone-Sereno P., Rosso M.-N., Engler G., de Almeida Engler J. 2012b. An immunocytochemical procedure for protein localization in various nematode life stages combined with plant tissues using methylacrylate embedded specimens. Phytopathology. 102 (10) : 990—996.

Yan Y., Smant G., Davis E. 2001. Functional screening yields a new beta-1.4-endoglucanase gene from *Heterodera glycines* that may be the product of recent gene duplication. Molecular Plant—Microbe Interactions. 14 (1) : 63— 71.

Yan Y., Smant G., Stokkermans J., Qin L., Helder J., Baum T., Schots A., Davis E. 1998. Genomic organization of four-1.4-endoglucanase genes in plant-parasitic cyst nematodes and its evolutionary implications. Gene. 220 (1—2) : 61—70.

Waetzig G. H., Sobczak M., Grundler F. M. W. 1999. Localization of hydrogen peroxide during the defense response of Arabidopsis thaliana against the plant-parasitic nematode *Heterodera glycines*. Nematology. 1 (7) : 681—686.

Williams V. M., Hussey R. S. 1996. Nematode pathogenesis and resistance in plants. Plant Cell. 8 : 1735—1745.

Wright D. J., Perry R. N. 2006. Reproduction, Physiology and Biochemistry. In: Plant nematology, Wallingford, CABI. 185—202.

Wubben M. J., Su H., Rodermel S. R., Baum T. J. 2001. Susceptibility to the sugar beet cyst nematode is modulated by ethylene signal transduction in Arabidopsis thaliana. Molecular Plant—Microbe Interactions. 14 (10) : 1206—1212.

Xue B., Hamamouch N., Li C., Huang G., Hussey R. S., Baum T. J., Davis E. L. 2013.The 8D05 parasitism gene of *Meloidogyne incognita* is required for successful infection of host roots. Phytopathology. 103 (2) : 175—181.

Zinovieva S. V. 1986. Relationship in plant-parasitic nematodes system from a phytoimmunity position. In: Questions of a biocenology of helminthes, Moscow, Nauka. 41—49 [In Russian].

Zinovieva S. V., Vasyukova N. I., Ozeretskovskaya O. L. 1990. Involvement of plant sterols in the system tomatoes. *Meloidogyne incognita*. Helminthologia. 27 (3) : 211—216.

Zinovieva S. V., Vasyukova N. I., Ozeretskovskaya O. L. 2004. Biochemical aspects of plant interactions with phytoparasitic nematodes: a review. Applied Biochemistry and Microbiology. 40 (2) : 111—119.

Zinovieva S. V., Vasyukova N. I., Udalova Zh. V., Gerasimova N. G. 2013. The participation of salicylic and jasmonic acids in genetic and induced resistance of tomato to (Kofoid and White, 1919). Biology Bullelin. 40 (3) : 297—303.